

Perinatal Nephropathies

by James E. Gibson*

The purpose of this paper is to review the development of the mammalian kidney and to assess the influence that various perinatal manipulations may have on the developmental process either morphologically or functionally. Immature kidneys in general have less functional capacity than adult kidneys and a low rate of glomerular filtration, perhaps related to renal blood flow, which appears to limit the disposition of a fluid or solute load. Tubular reabsorption is also limited leading to the urinary loss of glucose, amino acids, bicarbonate and phosphate. Although the relatively low function of the immature kidney is a normal part of development, its capacity to respond under conditions of stress may be less adequate than in adults. An additional concern is that a variety of perinatal manipulations, such as the incidental or accidental ingestion of a chemical, may lead to varying degrees of altered morphogenesis or functional development of the kidney. Chemically induced renal anomalies may be of several types, but in typical teratology experiments hydronephrosis may be the most frequent observation. The functional consequences of these renal malformations may be lethal or inconsequential or while an animal may be able to survive and develop normally in the presence of a renal malformation, it is possible that a stressful situation would unmask a functional malformation which could compromise survival. Thus, some renal abnormalities may be subtle enough to go unnoticed without experimental tests. Without such tests it is impossible to evaluate the effect of functional alterations on successful adaptation.

The development of the mammalian kidney begins early in gestation, and in most species this organ acquires some functional capacity before birth. However, birth does not mark the end of kidney development, for the kidney continues morphologic and functional maturation during the postnatal period. Inasmuch as the mature kidney can be affected by infectious disease or chemical injury it would be expected that the developing kidney may also be vulnerable to damage at various stages of development. Indeed, in mammals, a variety of morphologic and functional abnormalities of renal development have been recognized. Among the morphological changes are bilateral or unilateral renal agenesis, renal hypoplasia and renal dysplasia. The consequences of these abnormalities can range from the lethal effects of renal agenesis to the nearly nondetectable effects of some dysplasias.

The purpose of this paper is to review the development of the mammalian kidney and to assess the influence that various perinatal manipulations may have on the developmental process either morphologically or functionally.

Normal Morphologic Kidney Development

In the embryologic development of the mammalian kidney three successive sets of excretory organs appear; the pronephros, the mesonephros, and the metanephros. The metanephros remains as the permanent kidney. The pronephros is the first and simplest of the kidney systems to appear (8-9 somites or 3 weeks in human, 9 days in mouse). However, it appears only transiently during embryonic development and neither internal or external glomeruli nor collecting ducts develop. The pronephric duct remains to be utilized by the mesonephros kidney where the first excretory tubules are formed. Most likely the mesonephros (1-4 months in human; 9.5-10 days in mouse) functions as an interim kidney, itself degenerating as the permanent or metanephros kidney begins to function (metanephros development began fifth week in human, 23 somite embryo, day 11 in mouse; function 2 to 3 weeks later in human, day 14 in mouse). Mesonephric tubules

* Michigan State University, Department of Pharmacology, East Lansing, Michigan 48824.

develop and grow laterally until they contact the mesonephric duct, formerly the pronephric duct. The medial end of each tubule expands and becomes invaginated by the blood capillaries to form the glomerular capsule. At the end of the embryonic period the mesonephric kidney will have completely degenerated, leaving only the mesonephric duct which will be utilized as genital ducts in males.

The development of the metanephric kidney requires two anlagen: the ureteric bud and a metanephrogenic mass of mesoderm which will form a cap over the ureteric bud. Dilatation of the mesonephric duct leads to formation of the primitive renal pelvis; then calyces are formed near the middle of the pelvis. The pelvis divides into the major and minor calyces and collecting tubules grow out of the minor calyces. The stalk of the ureteric bud is the ureter and its dilated end forms the renal pelvis. The division of the pelvis into the major and minor calyces and the subsequent development of collecting tubules mark the beginning of the maturation of the metanephric kidney. Collecting tubules undergo subsequent branching, giving several generations of collecting tubules. Cell clusters in the metanephric cap differentiate and form small vesicles which in turn give rise to small tubules. Together the renal vesicles and the collecting tubules form a nephron. During growth, lengthening of the nephron results in the formation of the proximal convoluted tubules, a Loop of Henle and the distal convoluted tubule. Depending on the species, nephrons may or may not be completely developed at birth, although in humans and guinea pigs most nephrons have been formed at birth. Some of these nephrons complete their differentiation during infancy and continue to increase in size until adulthood. Thus, in these species hypertrophy and not an increase in the number of nephrons accounts for the growth of the kidney after birth.

Normal Functional Kidney Development

One well recognized feature of the newborn mammalian kidney is its relatively low level of function. However, this is not to say that the newborn kidney is nonfunctional. On the contrary, the function of the kidney *in utero* is essential for the formation of amniotic fluid and probably assists in regulating fetal water and solute composition. Among the factors which may account for the low functional activity of the *in utero* kidney may be relatively poor vascularization, which does not increase rapidly until after

birth. Some authors believe, however, that the low prenatal renal blood flow is not so much due to poor vascularization as it is to relatively high renal vascular resistance. Nevertheless, urine output during *in utero* life may be appreciable and related to the relatively poor reabsorptive process for sodium. Thus, in spite of a low glomerular filtration rate (GFR) the fetal kidney can have a high urine output as a result of a diminished or underdeveloped sodium reabsorption process (1).

In the transition from *in utero* life to the postnatal period, a kidney does not immediately acquire adult like function. The postnatal morphological and functional maturation of the mammalian kidney is slow and has been described as centrifugal. That is, functional maturation and structural maturation proceed from the inner toward the outer cortex. Thus, the first nephrons to form are those in the juxtamedullary area, and the last, the superficial ones. During growth there is a great increase in blood flow to the superficial cortical components relative to the juxtamedullary nephrons.

Studies of Spitzer and Brandis (2) in guinea pigs demonstrated that the increase in total kidney GFR which occurs during postnatal development is a consequence, during the immediate postpartum period, of an increase in filtration rate of the deep nephrons, whereas in 2-3 week old animals the main contributors to the rise in total kidney GFR are the superficial nephrons. Thus, these studies illustrate the concept of renal development from the center toward the periphery.

One aspect of the morphological development of the kidney that relates to functional maturation is the observation that the ratio between glomerular diameter and tubular volume is higher in the infant than later in life. This may be interpreted to mean that the immature kidney has a higher capacity to filter than to reabsorb or to secrete, possibly explaining why many newborn animals excrete a higher fraction of the filtered load of substances such as amino acids, phosphate, and bicarbonate. Thus, the generalization is made that tubular function of immature animals is markedly reduced in relation to the adult. Supporting this generalization are the observations of Hirsch et al. (3), who demonstrated using rabbit renal cortical slices *in vitro*, that *p*-aminohippuric acid (PAH) uptake is low at birth, but beginning at 2 weeks of age it increases rapidly until the maximum is reached at 4 weeks of age (Fig. 1). Histologically (Fig. 2), kidneys

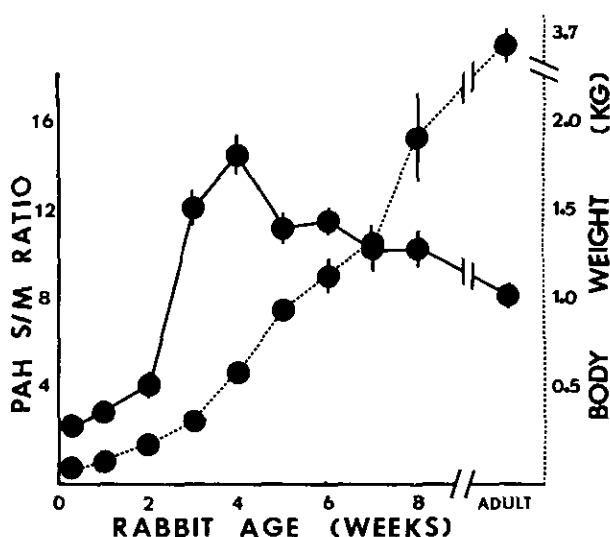


FIGURE 1. Effect of age (—) on accumulation of *p*-aminohippurate (PAH) by rabbit renal cortical slices and (---) on body weight. From Hirsch and Hook (4) with permission of the authors and The Journal of Pharmacology and Experimental Therapeutics.

from 2 week old rabbits had small densely cellular glomeruli and the cortical tubules were small with crowded nuclei, limited amounts of cytoplasm and inconspicuous tubular lumens. There were histologically undifferentiated or immature cells in the outer cortex. In contrast, by 4 weeks of age the renal tubules were nearly adultlike, both in size and appearance. Thus, histological development of the rabbit kidney correlated with its functional ability to accumulate PAH.

In vivo, immature animals also have a low extraction of PAH, that is, the extraction of PAH during one pass through the kidney may be on the order of 50–60% in newborns as compared to 85–90% in adult animals (5). Incomplete extraction of PAH by the immature transport processes in the tubules would partly explain this phenomenon, although another explanation related to the centrifugal pattern of renal development may also contribute to the understanding of this observation. A large portion of renal blood flow in immature animals bypasses secretory sites for PAH because in early life the nephrons which receive the largest portion of total renal flow are the juxtamedullary nephrons whose capillaries also provide the vasa recta. Medullary blood flow through the vasa recta does not contact the proximal tubules thus escaping PAH extraction. The data discussed earlier with relation to renal blood flow development confirms the validity of this hypothesis (6).

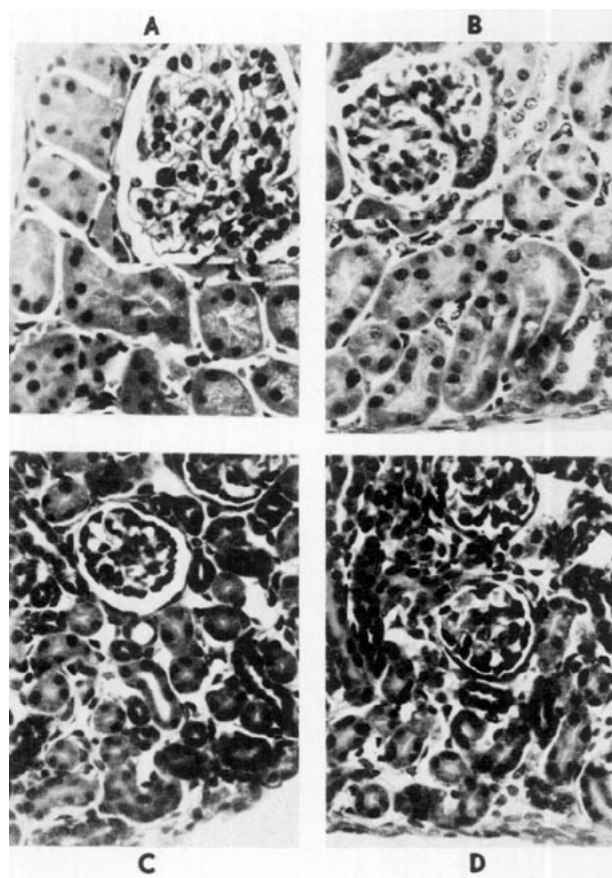


FIGURE 2. (A) Kidney from typical adult rabbit. Cortical tubules are full sized. Fully expanded, normally cellular glomerulus with only the occasional peripheral nucleus. $\times 460$; hematoxylin-eosin stain. (B) Kidney from typical 4-week old rabbit. Cortical tubules are adult size and contain ample cytoplasm. Glomerulus generally expanded and less cellular than in kidney from 2 week-old rabbit. $\times 460$; hematoxylin-eosin stain. (C) Kidney from typical 2 week-old penicillin-treated rabbit. Tubules and glomeruli are indistinguishable from those in upper right. $\times 460$; hematoxylin-eosin stain. (D) Kidney from typical 2 week-old saline-control rabbit. Glomerulus is small and densely cellular with prominent peripheral nuclei. Cortical tubules are small with crowded nuclei, limited amounts of cytoplasm, and inconspicuous tubular lumens. $\times 460$; hematoxylin-eosin stain. From Hirsch et al. (3) with permission of the authors and Proceedings of the Society for Experimental Biology and Medicine.

Functional maturation of the mammalian kidney may be summarized as follows. Immature kidneys in general have less functional capacity than adult kidneys. They have a better developed juxtamedullary than cortical area and an apparent glomerular preponderance. A low rate of glomerular filtration, perhaps related to renal

blood flow, appears to limit the disposition of a fluid or solute load. Tubular reabsorption is also limited leading to the urinary loss of glucose, amino acids, bicarbonate and phosphate. This relatively low function in the newborn is in no way detrimental to health of a normal animal. However, the capacity of the immature kidney to respond under conditions of stress may be less adequate than in adults.

Abnormal Morphological and Functional Kidney Development

Defects of kidney development are frequently encountered in teratology testing. In addition, renal abnormalities are common in live born human infants with estimates as high as 10% showing significant malformations of the urinary system. Many of these defects are obviously compatible with survival and they may only be recognized when secondary symptoms such as growth failure, anemia, uremia, rickets, pyelonephritis, or abdominal masses appear (7).

Our concern is that a variety of perinatal manipulations, such as the incidental or accidental ingestion of a chemical, may lead to varying degrees of altered morphogenesis or functional development of the kidney.

Absence of the kidney can arise in several ways as we might predict from our knowledge of the embryological development of the organ. The principal causes of renal agenesis relate to the anomalous growth of the ureteric bud, or when the ureteric bud fails to reach the metanephrogenic mass or a combination of these.

Simple hypoplasia is also a malformation of kidney development and may result from a decreased induction of the metanephric tissue such that the final kidney size is only one third its expected mass or less. Degenerative changes in previously normal tissue also contribute to some renal abnormalities. This type of response may occur with injury and is not a developmental lesion *per se* although a developing nephron may, in fact, be more susceptible to injury than a mature nephron.

The abnormal development of nephric and ductal structures is known as renal dysplasia. Dysplastic kidneys may be of any size or shape, and dysplasia may be cortical or medullary, total or partial, focal or segmental (8). The ultimate functional capacity of the kidney will depend on the type and extent of malformation.

In animal teratology studies, three types of renal anomalies have been associated with chemical administration: unilateral or bilateral renal agenesis, cystic kidneys, and hydronephrosis. Similar abnormalities occur in humans.

In man, polycystic kidney disease is probably a group of diseases for which there is incomplete understanding of the etiology and pathogenesis. Unfortunately, there is disagreement among pathologists and nephrologists regarding the classification of these diseases. However, defective embryonic growth and degeneration of formed nephrons seem to be responsible for the development of these anomalies.

In contrast to the problems encountered in classifying polycystic kidneys the abnormality known as hydronephrosis is easier to define: Hydronephrosis refers to dilatation of the renal pelvis usually combined with renal parenchymal compression. Some cases of hydronephrosis can be explained by intrauterine urinary tract obstruction. In fact, in infants with bilateral hydronephrosis, Usón et al. (9) estimated that 66% were due to obstruction.

One problem with hydronephrosis is that the boundary lines between physiologic and pathologic variations in renal pelvic size of fetuses are ill defined. Monie et al. (10) demonstrated that during normal development in the rat a temporary closure of the orifice of the ureters occurs in 16 to 19 day fetuses. To achieve normal development of the renal pelvis and ureters, the secretory function of the kidney during intrauterine life must be coordinated with the temporary closure and reopening of these orifices. If the orifices are closed for longer than normal then distention will take place above the point of temporary obstruction due to continuous urine formation. Hydronephrosis and/or hydroureter will result.

Woo and Hoar (11) noted that in rats late in gestation the renal papilla slowly and steadily increase in length and that the kidney parenchyma rapidly increase in weight. Thus, disparity in growth rate and delayed maturation may result in kidneys that have a large renal pelvis late in gestation. These dilated renal pelvises may inappropriately be called hydronephrotic. Woo and Hoar suggested (11) that this is only an "apparent hydronephrosis," since it commonly disappears shortly after birth.

Four causes of renal anomalies other than infectious disease may be listed: these are spontaneous, heritable renal anomalies, mechanically induced (obstruction) anomalies, anomalies asso-

ciated with nutritional deficiency states, and chemically induced anomalies.

There is evidence that the spontaneously occurring renal anomalies such as polycystic kidney and hydronephrosis are heritable traits in man and animals. Bernstein (12) reviewed the literature concerning heritable cystic disorders in humans but emphasized that not all cystic kidney diseases are genetically determined. Hydronephrotic kidneys occasionally occur in inbred breeding colonies of mice, and Collins et al. (13) described several cases of enlargement of renal pelvis in animals during routine autopsies. The affected kidneys appeared to have little functional parenchyma but interestingly the mice were remarkably free from signs of disease, even where advanced bilateral hydronephrosis was noted. Without autopsy it was not possible to identify affected animals. The most susceptible strain of mice for the spontaneous occurrence of hydronephrosis appears to be the BRVR strain (14). Histopathological studies suggested that the cause of hydronephrosis was ureteropelvic dyskinesia, that is, ureteral obstruction.

Experimental obstruction of the urinary tract during embryonic development results in two basic types of changes. First, if the ureteral ligation is made during the last half of fetal development, a hydronephrotic kidney similar to that seen after ureteral obstruction in mature animals occurs. On the other hand, if the urinary tract obstruction occurs during the first half of gestation, an entirely different result is obtained. Either unilateral or bilateral ureteral obstruction in the last half of gestation in lambs caused hydronephrotic kidneys which microscopically resembled the human dysplastic disease. However, ligation of one ureter early in gestation produced polycystic disease providing the contralateral kidney was removed (15). In rabbit fetuses, intrauterine ureteral ligation during the last third of gestation caused the development of hydronephrosis with marked changes in the renal parenchyma including dilation of the collecting ducts, tubules and Bowman's spaces. These changes were noted to occur very rapidly. In conjunction with dilation, severe cortical thinning occurred. The progression of the dilation appeared to progress from the pelvis, to the collecting ducts, to the nephron (16). The fetal and neonatal kidney specimens closely resembled the renal tissue of certain polycystic kidneys from human infants where there was a relatively uniform dilation of collecting tubules and where

obstruction was not considered a factor in pathogenesis. Thus, experimental intrauterine ureteral ligation techniques can be utilized to produce laboratory animal models of polycystic kidney disease as well as simple hydronephrosis.

Of greater concern, perhaps, in the present discussion of perinatal nephropathies, are those abnormalities related to the administration of an exogenous chemical or due to the specific exclusion of a nutritional agent. Diphenylamine and steroids may both produce severe polycystic kidney in the fetus. Long-term feeding of diphenylamine to the adult rat has been known for several years to cause cystic disease. Interestingly however, Safouh et al. (17) showed a loss in the concentrating ability of the kidney of diphenylamine fed rats long before morphological effects were evident. Close histological examination, however, usually revealed dilatations in the collecting ducts.

When diphenylamine was fed to pregnant rats during the last week of gestation at 2.5% in the diet, the kidneys of the newborn rats consistently showed dilatation of the renal tubules (7). Microdissection studies of the newborn kidneys demonstrated cystic lesions in the proximal portion of the nephron rather than in the collecting system as was noted for the adult rat. Thus, an experimental model of polycystic disease in the fetus may be produced by a chemical agent. However, diphenylamine itself may not be the causative agent, as purified diphenylamine does not produce these cysts, suggesting a contaminant of diphenylamine may be responsible for these lesions. A similar polycystic kidney disease reportedly is produced in newborn or weanling rabbits after injection of adrenal cortical steroids. However, these models do not as closely resemble the human polycystic disease as the diphenylamine model.

Hydronephrosis can also be induced by prenatal manipulation. In rats vitamin A administration on days 11 through 15 of gestation caused hydronephrosis while hypervitaminosis A has been associated with congenital renal anomalies in human infants (18). Folic acid and pantothenic acid deficiency in the rat and x-irradiation in the mouse also produced hydronephrosis.

Additional chemical compounds to which prenatal or neonatal animals have been exposed may cause renal abnormalities. Among these are drugs, pesticides and herbicides. One example of drug-induced renal abnormality is the "apparent hydronephrosis" in offspring of rats exposed to

methyl salicylate on days 10 and 11 of gestation. Methyl salicylate caused retarded renal development, particularly the growth of the renal papilla, and increased the number of both transient and permanent renal abnormalities in fetuses near term (11). Nearly 10% of the kidneys from treated animals showed gross dilatation of the renal pelvis and reduction of renal parenchyma at weaning, and this defect appeared to be permanent. At birth, the renal papilla of most treated kidneys were shorter than those in control kidneys demonstrating that methyl salicylate may reduce renal growth. In addition, there was a higher frequency of kidneys with absent papilla in the treated group than in the control. The renal papilla in both control and treated animals grew steadily from short to full length with advanced fetal and postnatal age and the fetal kidney weight doubled from day 19 to day 20 and increased over 60% from day 20 to day 21. It was suggested that this rapid growth in renal parenchyma coupled with a constant but slower increase in renal papillary length may result in a transitory formation of a large pelvis creating an appearance of hydronephrosis. Thus, in teratology experiments many "apparent" hydronephrotic kidneys may in fact be normal, and the renal growth only slightly delayed. To resolve this problem, "apparent hydronephrosis" must be distinguished from the permanent hydronephrotic kidney as was done in the methyl salicylate experiments.

Another example of chemically induced renal abnormalities was described by Courtney and Moore (19). They observed that the subcutaneous administration of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) to pregnant mice at a dose of 3 $\mu\text{g}/\text{kg}$ on gestational days 6 through 15 induced cleft palates and kidney anomalies in offspring. TCDD was a chemical contaminant of the herbicide 2,4,5-trichlorophenoxyacetic acid. The anomaly was described as "cystic kidneys," although this may have been an incorrect description as the lesion consisted of renal papillae which were markedly reduced in size or nonexistent with a large renal pelvis. These morphological changes may represent a retardation or absence of papilla development rather than a loss of already formed structure. In additional studies, TCDD, 3 $\mu\text{g}/\text{kg}$, administered on gestation days 10 through 13 to mice produced an incidence of 55% cleft palates. The incidence of kidney abnormalities was 95%. A level of 1 $\mu\text{g}/\text{kg}$ TCDD produced a 2% incidence of cleft palate, but the kidney abnormalities persisted at high levels

(20). In a series of cross fostering and reciprocal cross fostering experiments, it was found that there was a prenatal and postnatal component associated with TCDD treatment. Pups from TCDD treated mothers who nursed untreated mothers had virtually no kidney lesions on postnatal day 14. However, pups who nursed TCDD mothers but were born of untreated mothers had hydronephrotic kidneys. Therefore, exposure to TCDD treated females during the nursing period was a major factor in development of renal hydronephrosis. No signs of renal obstruction were noted. Association with the treated mother during the nursing period accounted for the highest incidence of hydronephrosis. It was suggested that pups were exposed to TCDD by its presence in milk. The failure to demonstrate progressive hydronephrosis postnatally in pups exposed only *in utero* is similar to the observation with the reversible lesions of methyl salicylate. The fact that these hydronephrotic kidneys can be maintained by the presence of an environmental contaminant is intriguing.

The consequences of severe renal malformation are obvious; that is, death or severe debilitation may result. An important question, however, relates to the functional capacities of moderately or slightly abnormal kidneys, or for that matter, the functional capacity of kidneys exposed to chemicals during development which show no apparent morphological change. This question has been approached to a small extent in experimental and clinical situations. For example, children affected with hydronephrosis were observed to have an impairment of maximal urinary concentrating capacity (21).

Some insight into the functional capacity of abnormal kidneys has been obtained in protein deficiency experiments. Kidneys from offspring of protein deficient rats are smaller and morphologically immature as compared to controls (22), and it was suggested that the reduction in kidney weight in protein deficient offspring was due to a reduction in nephron number (23). Functionally, offspring of protein deficient rats were less able to excrete a water or solute load from birth until 6 days after birth (24), and glomerular filtration rate was reduced. The inability of the newborn animals in protein deficiency experiments to excrete a water load was accompanied by an impairment in antidiuretic hormone responsiveness at 6 days but not at 13 days after birth. Partial reversal of these postnatal functional deficits could be obtained by increasing postnatal nutrition.

Another type of functional alteration in developing animals was demonstrated by Hirsch and Hook (25), when they treated pregnant rabbits with penicillin during the last half of pregnancy. Penicillin treatment resulted in an enhancement of PAH transport in renal cortical slices from the offspring at ages ranging from 1 day to 2 weeks (Fig. 3). Thus, PAH renal transport could be stimulated in the fetus by *in utero* administration of penicillin, demonstrating that the presence of an organic acid in the pregnant rabbit, and presumably the fetus, can stimulate maturation of tubular transport. The stimulatory effect of penicillin on PAH transport was reversible since when penicillin treatment was discontinued the stimulant for increased transport was removed and the transport capacity decreased to the normal levels for a particular age.

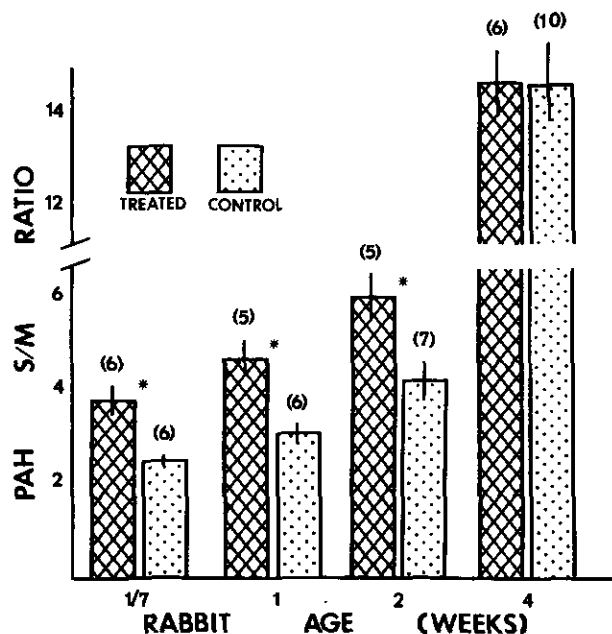


FIGURE 3. Accumulation of PAH by renal cortical slices (S/M) from rabbit offspring after daily IM injection of the pregnant doe during the last half of pregnancy with procaine penicillin G (60,000 I.U.). Newborn were studied at 1 day and 1, 2, and 4 weeks of age. Asterisks indicate those values that are significantly different from their respective controls ($p < 0.05$). From Hirsch and Hook (24) with permission of the authors and Science.

Since there is a correlation between functional development of the kidney and histological maturation, the stimulation of PAH accumulation by renal cortical slices might be expected to be associated with an enhanced histological matu-

ration of the kidney. However, this was not the case, as there was no apparent morphological difference between kidneys from penicillin-treated and control rabbits (Fig. 2). The importance of these observations is that in the absence of apparent morphological change an exogenous chemical brought about a functional change that was readily measured, as was the case with diphenylamine mentioned earlier. However, unlike the situation with the penicillin, diphenylamine-induced abnormalities became increasingly severe. Analyses of urea and sodium in the papillary tip of kidneys from diphenylamine treated rats after development of cystic kidneys showed a decrease in urea concentration and no change in sodium concentration. In addition, in the diphenylamine treated rats, there was an apparent correlation between the glomerular filtration rate and the severity of the morphological lesion, with GFR being reduced in the most severely involved group.

In relation to the functional capacity of polycystic or hydronephrotic kidneys, Agusta et al. (26) demonstrated in adult dogs a generalized decrease in metabolic processes in hydronephrotic kidneys. There was a shift from net glucose production to glucose utilization and simultaneously a shift in α -ketoglutarate net utilization to net production. These metabolic changes were obtained in kidneys whose ureters were obstructed for 2 or 6 weeks. Two weeks total ureteral obstruction produced a reversible injury whereas 6 weeks total obstruction produced irreversible injury. The hydronephrotic kidneys also had a significant decrease in oxygen consumption and carbon dioxide production. These impairments, even after correction for decreased renal blood flow, show that hydronephrosis has a greater metabolic derangement than ischemia alone.

Renal function studies in animals with kidney abnormalities induced during development are generally unknown. Presumably the functional deficits noted in abnormal adult kidneys would pertain to the abnormal kidneys of young animals. To test this hypothesis we have studied renal functional development in neonatal mice that received perinatal treatment with dinoseb, paraquat, and TCDD (J. E. Gibson and J. B. Hook, unpublished observations). Dinoseb, a herbicide, is the *sec*-butyl derivative of dinitrophenol, and produces a 30-40% incidence of apparent hydronephrosis in offspring of mice treated during gestation (27). Paraquat is a dipyrilidium herbicide

Table 1. Uptake of PAH by renal cortical slices of neonatal mice whose mothers were exposed to dinoseb during gestation.^a

Age, weeks	PAH uptake ^b		Impairment, %
	Control	Dinoseb	
1 (expt. 1)	2.33±0.23	1.73±0.15 ^c	26
1 (expt. 2)	4.65±0.40	3.21±0.21 ^c	31
2	6.23±0.87	3.26±0.31 ^c	48

^a Dinoseb, 15.8 mg/kg IP, was administered on days 10, 11, and 12 of gestation.

^b Renal cortical slices were incubated at 25°C under oxygen for 90 min. PAH uptake is the ratio of (¹⁴C-PAH in slice/³H-methoxyinulin in slice)/(¹⁴C-PAH in media/³H-methoxyinulin in media).

^c Significantly different from control ($p < 0.05$).

which does not produce hydronephrosis or morphological teratogenicity at all, even at doses nearly lethal to the mother (28).

Dinoseb caused a significant impairment in PAH uptake into renal cortical slices of offspring at 1 and 2 weeks of age (Table 1). Because of the small amount of renal tissue collected from young animals, it was necessary to express PAH uptake in terms of inulin space. Although dinoseb treatment produced an incidence of 30–40% hydro-

nephrosis detectable at birth, no grossly observable hydronephrosis was evident in these animals at 1 or 2 weeks of age. Thus, dinoseb produces "apparent hydronephrosis" which disappears by 1 to 2 weeks of age, although at this time PAH uptake is significantly reduced. The dinoseb effect on PAH uptake by renal cortical slices was also evident in 7 week old offspring from dinoseb-treated dams (Fig. 4). The data suggest that the transient renal cortical compression or the delayed growth of the kidney may delay maturation of PAH transport processes. Perinatal paraquat also diminished the ability of renal cortical slices from 2 week old treated animals to accumulate PAH (Fig. 5). Paraquat was administered in the drinking water of pregnant mice beginning at day 8 of gestation at 50 ppm. The treatment was continued throughout gestation, parturition and lactation. Paraquat did not affect maternal survival, number of live births, postnatal growth rate, or postnatal mortality of the offspring.

Another measure of kidney damage that we have used is ³H-phlorizin binding in renal cortical tissue. Phlorizin is a glycoside and may bind in part to sites responsible for glucose transport. We have hypothesized that alterations in phlori-

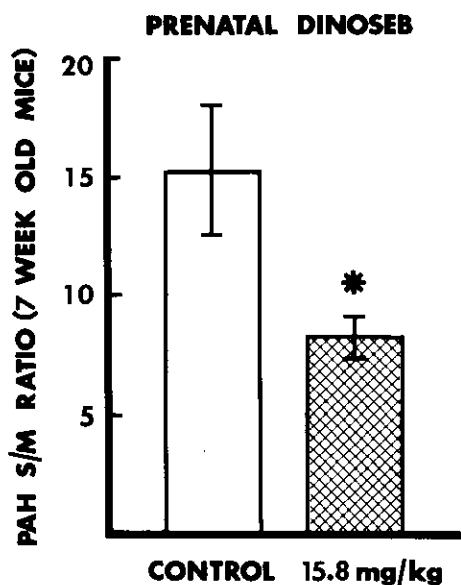


FIGURE 4. Accumulation of PAH by renal cortical slices (S/M) from Swiss-Webster mice offspring after daily administration of 15.8 mg/kg of dinoseb on gestational days 10–12. Pups were sacrificed, thin renal cortical slices were prepared and incubated for 90 min in a ¹⁴C PAH-³H methoxyinulin medium. Asterisk indicates a significant difference from control ($p < 0.05$).

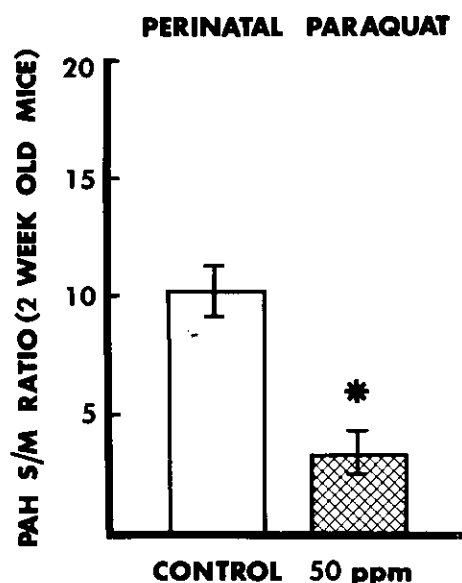


FIGURE 5. Accumulation of PAH by renal cortical slices (S/M) from mice offspring. Paraquat was added to the drinking water of pregnant Swiss-Webster mice from day 8 of gestation. Pups were sacrificed, thin renal cortical slices were prepared and incubated for 90 min in a ¹⁴C PAH-³H methoxyinulin medium. Asterisk indicates a significant difference from control ($p < 0.05$).

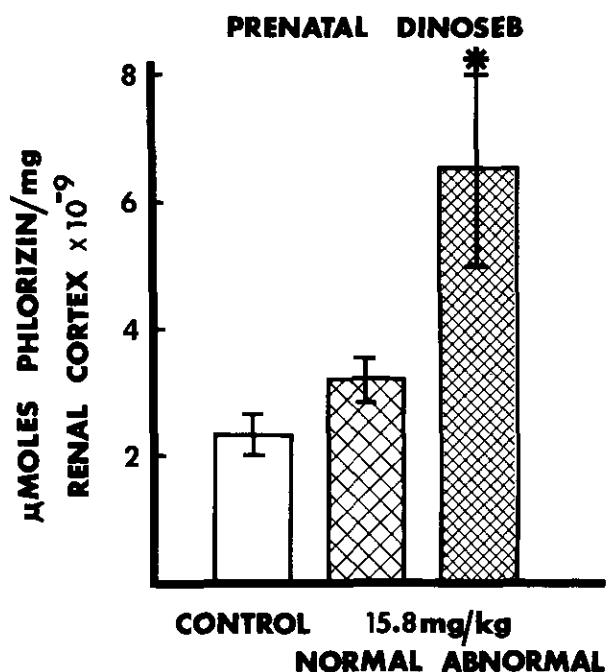


FIGURE 6. Phlorizin binding in renal cortical slices. Dinoseb was administered to pregnant Swiss Webster mice on days 10-12 of gestation. Pups, one to nine weeks of age, were examined for external morphological anomalies and then treated with $16 \mu M$ 3H -phlorizin IP 2 hr before sacrifice. Thin renal cortical slices were prepared and the μ mole 3H phlorizin/mg cortical tissue was determined. Asterisk indicates a significant difference from control ($p < 0.05$).

zin binding may be indicative of nephrotoxicity (29) primarily at the brush border where glucose is reabsorbed. The binding of phlorizin to the brush border and other sites is measured *in vitro* after *in vivo* administration. Phlorizin, 16μ mole/kg, was administered to offspring of mice treated prenatally with dinoseb or TCDD.

Prenatal dinoseb increased postnatal renal cortical binding of phlorizin (Fig. 6). However the effect was significant only in animals which showed observable external anomalies such as kinky tail or club foot. In animals without such anomalies there was no change in phlorizin binding.

TCDD prenatal treatment (2 or 4 μ g/kg day, days 10-15 gestation) produced different effects on phlorizin binding in renal cortical tissue. In 1 week old offspring there was a decrease in phlorizin binding (Fig. 7). Although it is too early to fully interpret these findings it is clear that binding changes do occur in offspring of animals administered teratogens during gestation.

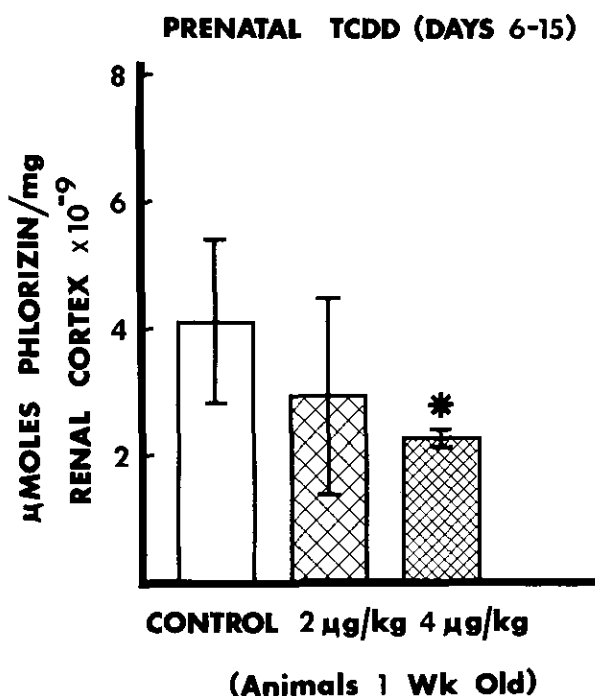


FIGURE 7. Phlorizin binding in renal cortical slices. TCDD was administered to pregnant Swiss Webster mice and the pups were then treated with $16 \mu M$ 3H phlorizin IP 2 hr prior to sacrifice. Thin renal cortical slices were prepared and μ mole 3H phlorizin/mg cortical tissue was determined. Asterisk indicates a significant difference from control ($p < 0.05$).

Summary and Conclusions

In this brief review the morphologic and functional development of the mammalian kidney has been described, and various mechanisms by which abnormalities of renal development may occur have been considered. Renal anomalies may be of several types, but in typical teratology experiments hydronephrosis may be the most frequent observation. In some cases the renal abnormalities may be of a transient nature, and no morphological defect will persist. These renal abnormalities may occur spontaneously, after mechanical obstructions such as ureteropelvic dyskinesia, after nutritional deficiencies, or after chemical exposure. A variety of chemical agents may be able to produce renal abnormalities. The functional consequences of these renal malformations may be lethal or inconsequential. However, while an animal may be able to survive and develop normally in the presence of a renal malfor-

mation, it is possible that a stressful situation would unmask a functional malformation which could compromise survival.

Techniques for studying functional development of the kidney are needed. Some methods for studying developing function have been mentioned in this presentation but additional thinking is needed. It is clear, however, that a teratology experiment cannot terminate with the morphological examination of the fetuses at term. Instead, studies of development need to include an assessment of organ functional maturation under conditions of chemical or other stress which may reveal functional abnormalities.

It is possible that some renal abnormalities are subtle enough to go unnoticed without experimental tests for detection. Without such tests it is impossible to evaluate the effect of functional alterations on successful adaptation. Research in this area has been neglected and should be expanded.

REFERENCES

- Alexander, D. P., and Nixon, D. A. The foetal kidney. *Brit. Med. Bull.* 17: 112 (1961).
- Spitzer, A., and Brandis, M. Functional and morphologic maturation of the superficial nephrons. Relationship to total kidney function. *J. Clin. Invest.* 53: 279 (1974).
- Hirsch, G. H., Cowan, D. F., and Hook, J. B. Histological changes in normal and drug-induced development of renal PAH transport. *Proc. Soc. Exptl. Biol. Med.* 137: 116 (1971).
- Hirsch, G. H., and Hook, J. B. Maturation of renal organic acid transport: Substrate stimulation by penicillin and *p*-aminohippurate (PAH). *J. Pharmacol. Exptl. Therap.* 171: 103 (1970).
- Calcagno, P. L., and Rubin, M. L. Renal extraction of *para*-aminohippurate in infants and children. *J. Clin. Invest.* 42: 1632 (1963).
- Edelmann, C. M., Jr., Pediatric nephrology. E. Mead Johnson award. *Pediatrics* 51: 854 (1973).
- Crocker, F. S., Brown, D. M. and Vernier, R. L. Developmental defects of the kidney. A review of renal development and experimental studies of maldevelopment. *Ped. Clin. N. Amer.* 18: 355 (1971).
- Bernstein, J. The morphogenesis of renal parenchymal maldevelopment (renal dysplasia). *Ped. Clin. N. Amer.* 18: 395 (1971).
- Uson, A. C., Cox, L. A., and Lattimer, J. K. Hydronephrosis in infants and children. *J. Amer. Med. Assoc.* 205: 323 (1968).
- Monie, I. W., Nelson, M. M., and Evans, H. M. Abnormalities of the urinary system of rat embryos resulting from transitory deficiency of pteroylglutamic acid during gestation. *Anat. Rec.* 127: 711 (1957).
- Woo, D. C., and Hoar, R. M. "Apparent hydronephrosis" as a normal aspect of renal development in late gestation of rats: The effects of methyl salicylate. *Teratology* 6: 191 (1972).
- Bernstein, J. Heritable cystic disorders of the kidney. The mythology of polycystic disease. *Ped. Clin. N. Amer.* 18: 435 (1971).
- Collins, G. R., Goodheart, C. R., and Henson, D. Spontaneous heritable hydronephrosis in inbred mice. I. Description, incidence and distribution of lesions. *Lab. Animal Sci.* 22: 333 (1972).
- Taylor, D. M., and Fraser, H. Hydronephrosis in inbred strains of mice with particular reference to the BRVR strain. *Lab. Animals* 7: 229 (1973).
- Beck, A. D. The effect of intra-uterine urinary obstruction upon the development of the fetal kidney. *J. Urol.* 105: 784 (1971).
- Thomasson, B. H., Esterly, J. R., and Ravitch, M. M. Morphologic changes in the fetal rabbit kidney after intrauterine ureteral ligation. *Invest. Urology* 8: 261 (1970).
- Safouh, M., Crocker, J. F. S., and Vernier, R. L. Experimental cystic disease of the kidney. *Lab. Invest.* 23: 392 (1970).
- Bernhardt, M. D., and Dorsey, D. J. Hypervitaminosis A and congenital renal anomalies in a human infant. *Obstet. Gynecol.* 43: 750 (1974).
- Courtney, K. D., and Moore, J. A. Teratology studies with 2,4,5-T and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Toxicol. Appl. Pharmacol.* 20: 396 (1971).
- Moore, J. A., et al. Postnatal effect of maternal exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). *Environ. Health Perspect.* 5: 81 (1973).
- Uttley, W. S., Paxton, J., and Thistlethwaite, D. Urinary concentrating ability and growth failure in urinary tract disorders. *Arch. Dis. Childhood* 47: 436 (1972).
- Potter, D., et al. Character of function and size in kidney during normal growth of rats. *Pediat. Res.* 3: 51 (1969).
- Allen, L. H., and Zeman, F. J. Kidney function in the progeny of protein-deficient rats. *J. Nutr.* 103: 1467 (1973).
- Hall, S. M., and Zeman, F. J. Kidney function of the progeny of rats fed a low protein diet. *J. Nutr.* 95: 49 (1968).
- Hirsch, G. H., and Hook, J. B. Maturation of renal organic acid transport: substrate stimulation by penicillin. *Science* 165: 909 (1969).
- Agusta, V. E., Panko, W. B., and Gillenwater, J. Y. Change in the *in vivo* metabolism of hydronephrotic canine kidneys. *Invest. Urology* 11: 379-385, 1974.
- Gibson, J. E. Teratology studies in mice with 2-sec-butyl-4,6-dinitrophenol (dinoseb). *Food Cosmet. Toxicol.* 11:31 (1973).
- Bus, J. S. Fetal toxicity and distribution of paraquat and diquat in mice and rats. *Toxicol. Appl. Pharmacol.* 33: 450 (1975).
- Ecker, J. L. Phlorizin binding to renal cortical tissue as an indicator of nephrotoxicity. *Toxicol. Appl. Pharmacol.* 33: 176 (1975).